1718

RESPONSES OF IMMATURE BLACKFLIES (DIPTERA: SIMULIIDAE) TO EXPERIMENTAL PULSES OF ACIDITY

OCTOBER 1991

Ontario
Environment
Environnement

ISBN 0-7729-8949-4

RESPONSES OF IMMATURE BLACKFLIES (DIPTERA: SIMULIIDAE) TO EXPERIMENTAL PULSES OF ACIDITY

Report prepared by:

C.M. Chmielewski
Trent University, Peterborough, Ontario

R.J. Hall
Environmental Sciences and Standards
Water Resources Branch
Ontario Ministry of the Environment

OCTOBER 1991

Cette publication technique
n'est disponible qu'en anglais.

Copyright: Queen's Printer for Ontario, 1991
This publication may be reproduced for non-commercial purposes
with appropriate attribution.

PIBS 1718

ABSTRACT

An investigation to determine the responses of immature blackflies to experimental pulses of acidity simulating snowmelt episodes was conducted. Five species of mature blackfly larvae were subjected to three or four day experimental pH depressions to ca. 4.0 or 3.5 in stream channels. Differences among species in response to acidity were observed. All species except *Simulium vittatum* and possibly *S. venustum* were able to tolerate pH depressions to ca. 4.0 while only *Cnephia dacotensis* and possibly *S. decorum* were able to tolerate pH depressions to ca. 3.5. The survival of the larvae (only late instars were tested) was significantly affected by the acidity while pupal survival generally was not. The rate of pupation, however, was decreased in the acidified channels resulting in decreased emergence there also. More abnormally formed pupae developed in the acidified channels, suggesting that pupation following snowmelt ensures survival of these species.

92-03442/

INTRODUCTION

The release of sulphur and nitrogen oxides into the atmosphere by the burning of fossil fuels has been designated the major cause for increased hydrogen ion content of precipitation in recent years (Galloway *et al.* 1976, Likens *et al.* 1979). Furthermore, this increased acidity of atmospheric deposition, both wet and dry, has been linked to the corresponding acidification of freshwater systems in Finland (Haapla *et al.* 1975), Norway (Gjessing *et al.* 1976, Wright *et al* 1976, Henriksen and Wright 1977), Sweden (Almer *et al.* 1974, Oden 1976, Hultberg and Wenblad 1980), northeastern United States (Cogbill and Likens 1974, Hendrey *et al.* 1980), and eastern Canada (Beamish 1976, Conroy *et al.* 1976, Jefferies *et al.* 1979). The most pronounced pH depressions in stream ecosystems occur as a result of hydrogen ion pulses during rain storms and especially during snowmelt. With the onset of snowmelt run-off, stream pH levels have dropped from circumneutral to as low as 3.9 in Ontario (Harvey 1980). These reductions can occur within hours and may alter benthic communities long before chronic acidification has been detected (Servos and Mackie 1986).

The effects of lowered stream pH on most stream biota are well documented, including such groups as mayflies, stoneflies, caddisflies and chironomids (e.g., Bell 1971, Sutcliffe and Carrick 1973, Hall *et al.* 1980, Raddum and Fjellheim 1984, MacKay and Kersey 1985, Hall and Ide 1987), but our knowledge of the effects of episodic pH depressions on blackflies (Diptera : Simuliidae) is decidedly scarce. Some studies have indicated that blackflies may be more tolerant of increased acidity than many of their stream co-habitants (Simpson *et al.* 1985, Sharpe *et al.* 1987, Hall *et al.* 1980, Bernard *et al.* 1989, Hopkins *et al.* 1988). In particular, Chmielewski (1989) reported an increase in blackfly emergence with increased acidity over a 50-year time span. Furthermore, MacKay and Kersey (1985) reported abundant blackflies in acid streams in the Dorset, Ontario area, and Hall *et al.* (1988) showed that *P. fuscum/mixtum* complex was not affected when exposed to short-term pulses of acidity in experimental transplants near Dorset. However, only a few studies have identified blackflies to species (Stoner *et al.* 1984, Simpson *et al.* 1985, Hall *et al.* 1988), and none have indicated toxicity responses at the specific level.

Therefore, a question of interest is how do blackfly immatures respond to short-term pulses of acidity similar to those encountered during snowmelt? Based on past surveys, the hypothesis to be tested in this research is that many blackfly species will be able to withstand short-term decreases in pH. Varying tolerances to low pH, explained in part by different life history strategies, are expected at the species level.

METHODS

The ability of different blackfly species to survive snowmelt acid pulses was tested by subjecting larvae to simulated snowmelt pH depressions. The artificial stream channels built by Serves and Mackie (1986) and located adjacent to the outflow of Plastic Lake, Haliburton County, Ontario (45° 11′ lat., 78° 50′ long.) were modified and used for the acidification experiments (Fig. 1).

To avoid using simuliid immatures with an acquired resistance to acidity, larvae were collected from Costello Creek, Algonquin Park, Ontario, a location that has not experienced extreme spring pH depressions (between 1984-86 the maximum spring pH depression was 5.9, Hall and Ide 1987) and has water quality, including annual pH range, similar to Plastic Lake Outflow (Findeis and Colman Taylor 1988).

A small concrete dam was constructed across Plastic Lake outflow with a 10-cm A.B.S. plastic overflow pipe leading to reservoir #1 (R1, approximately 20 l) 5 m downstream and then to the wooden plasticized channel system 5 m farther downstream. Here, the pipe divided, filling two larger reservoirs (R2 and R3) of approximately 700 l each. All three reservoirs had overflows. Reservoir 3 was acidified by mixing 1 N sulphuric acid with stream water via a peristaltic pump. This reservoir had a mixing cup and baffles to ensure complete mixing of the acid. Reservoir 2 contained a reference supply of stream water. One 7.3-cm manifold from the control and one from the acid reservoir supplied three flow-through channels (each 3.1 m x 28 cm x 45 cm). Water depth was approximately 5 cm. Mean discharges for the three experimental runs were approximately 120 mL/s, 160 mL/s, and 390 mL/s, respectively. Acidified and control channels were chosen randomly and were different for each experiment.

Chambers were designed to contain larvae in the stream channels while allowing water to flow unimpeded through the channels (Fig. 1). Two rectangular holes were cut from opposite sides of 500-mL cylindrical, plastic containers and replaced with Nitex netting (430-μm mesh for the first experiment and 250-μm for the second and third). To catch emerging adults, a 6-mm funnel was inverted and glued to a lid with a circular hole of the

same size. The small end of the funnel protruded through a hole cut in the bottom of a 500-mL Nalgene bottle and was also glued. The emergence chamber was completely sealed so that emerging adults would fly through the funnel and drop into the Nalgene bottle containing 70% alcohol.

Rocks, sticks and trailing vegetation with attached larvae were taken from all areas of Costello Creek to collect the most diverse selection of species as possible and reduce the amount of manipulation. They were then transferred to aerated coolers filled with stream water. Larvae were carefully transferred to emergence chambers (50 per chamber) with forceps suspended with a drop of water between its arms and then placed into wooden streamside channels within 3-4 hours. Larvae greater than *ca.* 0.7 cm long were selected because smaller larvae could escape through the mesh of the chambers. Larvae were not identified at this point to minimize handling effects. Species distribution among the chambers was not known but assumed to be random.

Three runs of two experiments each were conducted (Table 1). Starting dates were chosen on or before peak emergence for the greatest number of species, based on eleven years of emergence data from Costello Creek (Davies 1950, Hayton 1979). Runs 1, 2 and 3 were started on 29 April, 19 May, and 12 June, 1987, respectively. The first run consisted of 4 days at pH 4.0 and one day at pH 3.5, the second consisted of 3 days at pH 4.0 and one day at pH 3.5 and the third consisted of 3 days at pH 4.0 and one day at pH 3.5.

At the beginning of each experiment three emergence chambers were randomly placed in each of six channels (6 channels x 3 chambers/channel x 50 larvae/chamber = 900 larvae). Larvae were allowed to acclimate overnight in the channels. Dead larvae were removed the following morning and discarded. The pH was then decreased to 4.0 or 3.5 in treatment channels within two hours and maintained at that level until the end of the experiment. The chambers were checked daily and dead blackflies were removed, counted and preserved in 95% ethanol. At the end of each experiment the remaining emergence chambers were removed from the channels, and the live and dead blackflies preserved.

Back-transplants back to Costello Creek were initiated to determine whether handling had adverse effects on blackfly survival. Six emergence chambers (50 larvae in each) were returned to Costello Creek on the same day of the first collection of run 2 (19 May) and 3 (12 June). Three plastic pails with large-mesh (*ca.* 1 cm) screened walls on opposite sides of the pails were placed in the stream and two emergence chambers placed in each. Both chambers were checked for live and dead larvae when the second collection per run was made (3 days later). One chamber was removed at this time and the live and dead blackflies counted. Another chamber containing blackflies that had been collected that day and returned to the creek from the laboratory was placed in each pail. Both chambers were left until the end of the Run (3 more days) when larvae were removed and counted.

The temperature and pH of Costello Creek were taken at the time of each collection, and maximum and minimum stream temperatures were recorded daily. pH of one control channel and one acidified channel was monitored at least hourly during the day and bi-hourly overnight twice during Run 3. Discharge of each channel was checked on a regular basis (usually once per day), except in Run 2 when it was recorded only once. Discharge was measured by catching the entire flow of a channel in a graduated cylinder for 10 seconds. Water samples (one from each channel) were taken at the beginning and end of each run for chemical analysis of pH, alkalinity, total aluminum, conductivity, calcium, dissolved inorganic carbon, dissolved organic carbon, and sulphate. At the end of each run, water samples were taken (from one acidified channel and one control channel) for analysis of cadmium, copper, lead, and zinc. All water samples were analyzed at the Ontario Ministry of the Environment Research Centre, Dorset, Ontario following standard procedures (LaZerte 1984, Locke and Scott 1986).

Blackflies were identified using keys of Davies *et al.* (1962) for the adults and Wood *et al.* (1963) for the larvae and pupae. No attempt was made to separate the species cytotypically. Consequently, the names used refer to morphospecies only. At time of identification, the head capsule of each larvae was measured at its widest dorsal point using an ocular micrometer (accuracy of 0.025 mm).

Due to variation in conditions among runs, each experiment was analyzed separately with qualitative comparisons made between experiments. The arsine-transformed, unpooled survival data, weighted for number of larvae of each species in an emergence chamber, were analyzed with a nested analysis of variance to determine among-chamber variability in survival within each species. Since the among-chamber variation in survival was not significant (P = .10 to .94), the number of live and dead larvae of each species were pooled for all emergence chambers within an experiment. Subsequently, contingency table analysis (2x2) was performed to determine if acidification had an effect on survival of each species. Additionally, each species was subdivided into life stages, and survival of each stage was analyzed by 2x2 contingency tables. Finally, to determine if the response to acidity was the same for all species in an experiment, repeated measures analysis of variance was performed on arsine-transformed, unpooled, weighted survival data. The alpha level chosen for all statistics was 0.05.

RESULTS

Channel Conditions

The conditions in the channels were generally amenable to blackfly habitation. Larvae back-transplanted to Costello Creek had a lower survival rate than those in control channels in two out of the three acidification experiments in the artificial channels (Chmielewski 1989). Thus, it appears the control water from Plastic Lake outflow was a favourable substitute for Costello Creek water but the manipulations may have been a major factor in the low survival of some of the control blackflies (Table 2).

Differences in minimum and maximum temperatures of reservoir water between each run reflect a warming trend in the stream as spring progressed. Water temperature in Run 1, which began on 29 April shortly after snowmelt, ranged between 3° and 23° C. Water temperature during Run 2, which began 19 May, ranged from 10° to 18° C. The weather was much cooler and wetter in the second half of May than the first. During Run 3 (starting on 12 June) the weather was comparably warmer as reflected in the higher range of reservoir temperature (11° to 26° C).

Discharge was well replicated among channels in experiments 2 (160 mL/S) and 3 (390 mL/S). In Run 1 a large part of the variation in discharge (85%) occurred between acidified and control channels, with discharge rate of control channels (128 mL/s, s.e. = 1.0) being higher on average (nested ANOVA, P = 0.001) than acidified channels (113 ml/s, s.e. = 2.0). As blackflies are known to have specific flow rate requirements (Hopkins *et al.* 1988), the differences in flow may be, in part, responsible for differences in survival between the control and acidified channels on Run 1.

A general trend during most experiments was that Al, Ca, SO_4, and conductivity increased more in acidified than control channels (Table 3). Alkalinity was depressed in acidified channels of both Run 1 and 3. Observed effects of acidification on water quality were less pronounced in Run 2 because of difficulty encountered in maintaining

the pH at depressed levels in the second experiment of the run. Because of these difficulties the results of that experiment were discarded. Both dissolved inorganic and organic carbon increased slightly in acidified and control channels during each run (except for DIC of Run 3 which decreased in both acidified and control channels, Table 3). Of the four metals measured at the end of each run (Table 3), only zinc had consistently higher concentrations in acidified channels. Copper may have been mobilized in acidified channels, and cadmium and lead were always at or near minimum detection limits.

Development and Survival of Blackflies under Acidic Conditions

Because of the random nature of the collection and distribution of the blackflies, the identity and numbers of species tested were not known until after the experiments were concluded. The results for a particular species in an experiment were not presented if the survival of the species in the control channels was less than 40% or if there were too few individuals of that species to draw reasonable conclusions. Five species were collected in sufficient numbers for meaningful analysis. These species were *Cnephia dacotensis* (Dyar and Shannon), *Simulium decorum* Walker complex, *S. venustum* Say complex, *S. verecundum* Stone and Jamnback complex, and *S. vittatum* Zetterstedt complex (Table 2).

Presently there are no reliable characters to separate pupal stages of *S. tuberosum* (Lundström) complex, *S. venustum* and *S. verecundum* (Stone and Jamnback 1955, Davies *et al.* 1962). In addition, in this study there were morphological intergrades between the larvae of 24 *S. venustum* and *S. verecundum*. Although the numbers of these inseparable individuals were relatively few, they were presented as a separate group for the sake of completeness. This group may include pupae of *S. tuberosum* (because small numbers of the species were present) but is likely composed mostly of *S. venustum* as this was otherwise the most numerous of the three species.

Several patterns emerged from contingency table analysis of the acidification experiments (Table 2). The first was that survival of one species, *C. dacotensis*, was unaffected for four days at either pH 4.0 or 3.5 (Run 1). The

second pattern, exhibited by *S. verecundum*, was that pH 4.0 had no effect on survival for 3 days (Run 2) and pH 3.5 caused reduced survival (Run 3). A third response was significantly reduced survival at both pH levels for 3 days as exhibited by *S. vittatum* (Run 3). Finally, *S. venustum* and *S. decorum* yielded somewhat conflicting results. At pH 4.0 the survival of *S. venustum* was unaffected in one 3-day experiment (Run 2) but reduced in another 3-day experiment with borderline significance (Run 3) in one 4-day experiment (Run 1). At pH 3.5 for 3 days its survival was significantly reduced in the acidified channels (Run 3). The survival of *S. decorum* was unaffected at pH 4.0 for 3 days (Run 2 and 3). At pH 3.5 its survival was unchanged when subjected for 4 days (Run 1), but it was significantly affected during a 3-day experiment (Run 3).

There were no differences observed when survival in acidic conditions was compared among species within an experiment. Only in the two experiments of Run 3 were there representatives of each species in each emergence chamber to satisfy the requirements of the repeated measures analysis of variance that there be no missing values (Hand and Taylor 1987). In the pH 4.0 experiments the results of repeated measures analysis of variance did not agree completely with contingency table results. The latter indicated a significantly lower survival of *S. venustum* ($0.025 < P < 0.05$) and *S. vittatum* ($0.001 < P < 0.005$) in acidified as opposed to control channels, whereas repeated measures analysis of variance indicated that, among species, blackfly survival between acidified and control channels was not significantly different (although the significance level was borderline at $P = 0.0562$). Accordingly, since no species was significantly affected by acidity at pH 4.0, repeated measures analysis of variance found no significant differences in tolerances to acidity among species ($P = 0.4147$). Alternatively, at pH 3.5 in Run 3, the results of repeated measures analysis of variance agreed with results of contingency tables in that among species there was a significant difference in survival between those in control and acidified channels ($P = 0.0001$). Furthermore, repeated measures analysis of variance also indicated that there was no difference among species in the increase in mortality correlated with acidity ($P = 0.3532$).

During the experiments the blackfly larvae matured at various rates (Table 4). By the end of each experiment the majority of blackflies were still in the larval stage with the size of the majority decreasing with each successive experiment. Analysis of variance followed by Tukey's Multiple Range tests on the larval head capsule widths

also indicated that larval size increased in successive experiments in all species except *S. venustum* (P = 0.0009 for *S. verecundum*, P = 0.0001 for all others). It exhibited a bimodal increase, possibly indicating the presence of two generations (*S. decorum* was the only species that displayed a significantly greater mean head capsule width in the acidified channels as opposed to control channels [P = 0.0156]). The rate of pupation and adult emergence was greatest for most species in the two experiments of Run 3 in early June.

There were three important findings on stage-dependent sensitivity to acidity (Table 4). First, if the survival of a species was found to be significantly reduced under acidic treatment, the stage that was responsible for the difference was almost invariably the larval stage. In other words, once the blackflies had pupated, the acidity did not affect their survival. In fact, pupal survival was significantly greater in acidified channels at least twice (Table 4). Second, the percentage of pupation overall was decreased in the acidified channels in all species but *S. venustum* (but many of its pupae were included in the inseparable group of *S. tuberosum, S. venustum* and *S tuberosum*). Accordingly, there was also a smaller percentage of blackflies emerging from the acidified channels. Third, many individuals that had attempted pupation in the experiments were malformed. The head and appendages were disfigured to varying degrees and the cocoon was often incomplete or missing. These "half pupated forms" occurred most often in the acidified channels. Quite unexpectedly, however, their survival was often numerically greater (although significantly greater only once) in acidified than in control channels (see Chmielewski 1989).

DISCUSSION

The present study has extended our knowledge of the uncommon tolerance of blackflies to reduced pH but has also indicated that there are differences in acid tolerances among species and life stages (larvae, pupae and adult) of blackflies. The survival of several species of blackflies at a pH of *ca.* 4.0 in the present study is in agreement with other studies that have found blackflies to be more acid tolerant than other taxa. Surveys of acidified and non-acidified streams have revealed a distinct tolerance of blackflies to depressed pH relative to other stream invertebrates (Minshall and Minshall 1978, Sutcliffe and Carrick 1973, Stoner *et al.* 1984, Simpson *et al.* 1985, Sharpe *et al.* 1987). More specifically, *Simulium* spp. drift rates were not affected in a 12 hour whole stream acidification from a pH of 7.0 to 5.9, whereas drift rates of other taxa increased markedly (Bernard *et al.*1989). *Simulium* spp. drift rates were also found to be unaffected by 6 to 8-hour depressions of pH to *ca.* 5.0 in naturally colonized stream channel experiments (Cooper *et al.* 1988, Hopkins *et al.* 1988). Even in a study where individuals of *Prosimulium fuscum/mixtum* complex (Fries) were transplanted from streams of pH 6.2 to 4.5 for 4 days, survival was nearly complete (Hall *et al.* 1988). In the same study in a 10-day transplant from a stream of pH 5.6 to one of 4.5 an 80% survival rate for blackflies was obtained (Hall *et al.* 1988). It was not until stream pH was depressed to 4.0 for 5 months that a definite negative response to acidity was detected in increased *Prosimulium* spp. drift (Hall *et al.* 1980). A similar pH depression to 4.0 in the present study also elicited a negative response in *Simulium vittatum* after 3 days but at least 3 other species, *Cnephia dacotensis, Simulium decorum, S. verecundum,* and possibly *S. venustum*, were unaffected at this pH level. Further testing of the blackflies revealed even more specific differences as *C. dacotensis* and possibly *S. decorum* were the only species able to tolerate a pH reduced to *ca.* 3.5. Because of the differences found among species in their response to acidity, the importance of species level identification in invertebrate toxicity studies cannot be stressed enough.

Smaller size classes of other invertebrates are often the most sensitive to experimentally lowered pH levels (Allan and Burton 1986, Allard and Moreau 1987). Depressed emergence of stream invertebrates exposed to decreased pH has also been observed (Bell 1971, Hall *et al.* 1980, Zischke *et al.* 1983). Zischke *et al.* (1983) and Bell (1971)

concluded that the emerging insect is the most sensitive stage, but their conclusions may have been premature. In stream channel acidification studies where organisms were allowed to colonize channels naturally (Zischke *et al.* 1983), it was impossible to monitor survival of early stages. Bell (1971) used only mature larvae of stream insects in his laboratory bioassays and therefore should not have concluded that the emerging insect was the most sensitive stage without having tested early stages. Unfortunately, smaller blackfly instars could not be included in this study either because smaller mesh sizes could not be used due to problems with rapid siltation. Thus, no definitive conclusions can be made as to which life stage is the most sensitive. However, some insights into stage-dependent sensitivity of blackflies are offered. Although there was little emergence data in these studies, there was a slightly decreased emergence observed in some species in acidified channels, results which support those of Bell (1971), Hall *et al.* (1980), and Zischke *et al.* (1983). However, observation of decreased pupation of some species in acidified channels (*Cnephia dacotensis, Simulium decorum, S. venustum/verecundum, S. vittatum*, Table 7) leads to the more precise conclusion that, because fewer blackflies pupate in acidified conditions, emergence may be decreased.

To date, no one has attempted to rear blackflies in outdoor stream channels. Difficulty in raising blackflies has been attributed to susceptibility of stream insects to accumulated ammonium wastes, and narrow hydrochemical, temperature, current and food tolerances (Edman and Simmons 1985). However, it is unclear why three- or four-day survival of control blackflies in this study was sometimes low, compared to laboratory studies lasting up to several months (Edman and Simmons 1985), although it is possible that the blackfly mortality observed in control channels and back-transplants may be more similar to their natural mortality during development and emergence. There are several factors that may have influenced survival. Water temperature has been shown to be a critical factor affecting larval blackfly distribution (Ross and Merritt 1978) and may not have been optimal during rearing, especially for some of the early spring species that prefer lower temperatures. Their food requirements appeared to be satisfied, as their guts were always full. Although there are no indications of such, perhaps there was a critical difference that was not monitored between water from Plastic Lake Outflow and Costello Creek . Lowered rates of discharge have also been found to be an additional stress confounding interpretation of results from acidification testing of blackflies (Hopkins *et al.* 1988). It may not have been

optimal for all species at all times but, more importantly, the variation in discharge between acidified and control chambers in Run 1 may have been, in part, responsible for the significantly lower survival of *S. venustum* in the acidified channels. *C. dacotensis* did not seem to be affected in the same experiment. Perhaps the most important point to consider is that since there were three to five species being raised simultaneously, it was impossible to meet all of their specific requirements. However, any advantages one species could have had over another were taken into account by repeated measures analysis of variance because it was difference in survival between control and treated of each species that was compared, not just survival of the treated.

The increased number of malformed pupae in acidified channels clearly indicates presence of a severe physiological stress on blackflies when subjected to depressed pH levels. No studies have assessed direct toxicity of excess hydrogen ions on immature blackflies, thus it is difficult to point out the exact physiological causes of malformations or of mortality. However, Hall *et al.* (1988) have monitored cation concentrations of blackflies cross-transplanted from a site at pH 6.5 to one of pH 4.2. They reported a significant decrease in whole-body calcium and sodium concentrations, which may indicate a change in ionoregulation with decreased pH (Hall *et al.* 1988). The occurrence of abnormal, half-pupated forms are thought to be the result of less than optimal rearing conditions.

The stage at which a species is present when stream pH depressions occur in an important aspect of life history strategies (see Ross and Merritt 1987). Most of the blackflies tested from Costello Creek overwinter in the egg stage (Davies *et al.* 1962), and hatching does not occur until mid-April or later in most species (Davies *et al.* 1962), generally after snow-melt. Only a few early spring species may overwinter as larvae including *C. dacotensis* and *S. vittatum*, although there are conflicting reports for both (see Hayton 1979). These are the only species of blackfly included in this study whose larvae may have experienced spring snowmelt. More importantly, those tested were survivors of the real snowmelt, and *C. dacotensis* is deemed the species (of those tested) most tolerant to pH depressions. Perhaps because the majority of blackflies in temperate zones do overwinter as eggs and miss snowmelt as larvae, they are more tolerant than other taxa that may overwinter as larvae. The generally high larval survival in acidified channels in this study suggest that blackfly larvae may be able to survive

pH depressions following rain events subsequent to snowmelt. Since the pupation process itself appeared to be negatively affected by low pH, insects surviving in areas of high acidic deposition need to delay pupation until after snowmelt. In contrast, those species that are more tolerant to pH depressions can occupy early-season habitats that are not amenable to more pH-sensitive species. Our hypothesis, then, was supported by data for some species and refuted based on others. Life history strategies clearly play a dominant role.

ACKNOWLEDGEMENTS

Support for this research was provided by Ontario Graduate Scholarships and the Ontario Ministry of the Environment to C.M. Chmielewski. Many members of the staff at the Dorset Research Centre assisted with various aspects of this study. In particular, we thank J.G. Findeis, L. Heintsch, G. Little, T. Champoux, and R. Gilbert for able assistance in the field and to J. O'Hara Hines of the University of Waterloo for her competent statistical advice. M. Berrill, J.G. Chmielewski, L.D. Corkum, D.M. Davies, E. Nol and C. Metcalf made useful comments on early drafts of this manuscript.

REFERENCES

ALLAN, J.W. and T.M. BURTON. 1986. Size dependent sensitivity of three species of stream invertebrates to pH depression. In: Impact of acid rain and deposition on aquatic biological systems. ASTM STP 928. B.G. ISOM, S.D. DENNIS, and J.M. BATES (eds.) American Society for Testing and Materials. Philadelphia, pp 54-66.

ALLARD, M. and G. MOREAU. 1987. Effects of experimental acidification on a lotic macroinvertebrate community. Hydrobiologia. 144: 37-49.

ALMER, B., W. DICKSON, C. EKSTORM, E. HORNSTORM and U. MILLER. 1974. Effects of acidification on Swedish Lakes. Ambio. 3: 30-36.

BEAMISH, R.J. 1976. Acidification of lakes in Canada by acid precipitation and the resulting effects on fishes. Water, Air and Soil Pollut. 6: 501-514.

BELL, H.L. 1971. Effect of low pH on the survival and emergence of aquatic insects. Water Res. 5: 313-319.

BERNARD, D.P., W.E. NEILL, and L. ROWE. 1989. Impact of mild experimental acidification on short-term invertebrate drift in a sensitive British Columbia stream. Hydrobiologia, in press.

CHMIELEWSKI, C.M. 1989. Responses of blackflies (*Diptera: Simuliidae*) to acidity using stream channels and emergence surveys spanning 50 years. M.S. Thesis. Trent University, Peterborough, Ontario.

COGBILL, C.V. and G.E. LIKENS. 1974. Acid precipitation in the northeastern United States. Water Resour. Res. 10: 1133-1137.

CONROY, N., K. HAWLEY, W. KELLER and C. LAFRANCE. 1976. Influences of the atmosphere on lakes in the Sudbury area. J. Great Lakes Res. 2 (Suppl. 1): 146-165.

COOPER, S.D., K. KRATZ, R.W. HOLMES and J.M. MELACK. 1988. An integrated watershed study: An investigation of the biota in the Emerald Lake system and stream channel experiments. Report for California Air Resources Board, Contract A5 139-32.

DAVIES, D.M. 1950. A study of the blackfly populations of a stream in Algonquin Park, Ontario. Trans. R. Can. Inst. 59: 121-160.

DAVIES, D.M., B.V. PETERSON and D.M. WOOD. 1962. The black flies (*Diptera: Simuliidae*) of Ontario. Part 1: Adult identification and distribution with descriptions of six new species. Proc. Entomol. Soc. Ont. 92: 71-154.

EDMAN, J.D. and K.R. SIMMONS. 1985. Rearing and colonization of black flies (*Diptera: Simuliidae*). J. Med. Entomol. 22(1): 1-17.

FINDEIS, J.G. and M. COLEMAN TAYLOR. 1988. Chemical and biological data summary for Plastic Lake outflow acidification experiments (spring and autumn, 1982). Ontario Ministry of the Environment DR 8-2, 107 pp.

GALLOWAY, J.N., G.E. LIKENS and E.S. EDGERTON. 1976. Hydrogen ion speciation in the acid precipitation of the northeastern United States. Water Air and Soil Pollut. 6: 423-433.

GJESSING, E.T., A. HENRIKSEN, M. JOHANNESSEN and R.F. WRIGHT. 1976. Effects of acid precipitation on freshwater chemistry. In: Impact of acid precipitation on forest and freshwater

ecosystems in Norway. F.W. BRAEKKE (ed.) Research Report 6/76, SNSF Project, Oslo, Norway. pp. 64-85.

HAAPLA, H., P. SEPPONEN and E. MESKUS. 1975. Effects of spring floods on water acidity in the Kliminkijoki area, Finland. Oikos 26: 21-31.

HALL, R.J. and F.P. IDE. 1987. Evidence of acidification effects on stream insect communities in central Ontario between 1937 and 1985. Can. J. Fish. Aquat. Sci. 44: 1652-1657.

HALL, R.J., G.E. LIKENS, S.B. FIANCE and G.R. HENDRY. 1980. Experimental acidification of a stream in the Hubbard Brook Experimental Forest, New Hampshire. Ecology. 61: 976-989.

HALL, R.J., R.C. BAILEY and J. FINDEIS. 1988. Factors affecting survival and cation concentration in the blackflies *Prosimulium fuscum/mixtum* and the mayfly *Leptophlebia cupida* during spring snowmelt. Can. J. Fish. Aquat. Sci. 45: 2123-2132.

HAND, D.J. and C.C. TAYLOR. 1987. Multivariate and repeated measures analysis of variance. Chapman and Hall N.Y.

HARVEY, H.H. 1980. Widespread and diverse changes in the biota of North American lakes and rivers coincident with acidification. In: Proceedings of an International Conference on the Ecological Impact of Acid Precipitation. D. DRABLOS and A. TOLLAN (eds.) SNSF Project, Sandefjord, Norway. pp. 93-98.

HAYTON, A. 1979. The age structure and population dynamics of some black-flies in Algonquin Park, Ontario. MSc. Thesis, University of Waterloo, Waterloo, Ontario.

HENDRY, G.R., J.N. GALLOWAY, S.A. NORTON, C.L. SCHOFIELD, D.A. BURNS and P.W. SCHAFFER. 1980. Sensitivity of the eastern United States to acid precipitation impacts on surface waters. In: Proc. Int. Conf. Ecol. Impact Acid Precip. D. DRABLOS and A. TOLLAN (eds.) Norway SNSF Project pp. 216-217.

HENRIKSEN, A. and R.F. WRIGHT. 1977. Effects of acid precipitation on a small acid lake in southern Norway. Nord. Hydrol. 8: 1-10.

HOPKINS, P.S., K.W. KRATZ and S.D. COOPER. 1988. Effects of an experimental acid pulse on invertebrates in a high altitude Sierra Nevada stream. Hydrobiologia, in press.

HULTBERG, H. and A. WENBLAD. 1980. Acid groundwater in southwestern Sweeden In: Proc. Int. Conf. Ecol. Impact Acid Precip. D. DRABLOS and A. TOLLAN (eds.) Norway SNSF Project pp. 220-221.

JEFFRIES, D.S., C.M. COX and P.J. DILLON. 1979. Depression of pH in lakes and streams in central Ontario during snowmelt. J. Fish. Res. Board Can. 36: 640-646.

LAZERTE, B. 1984. Forms of aqueous aluminum in acidified catchments of Southern Ontario: A methodolocical analysis. Can. J. Fish. Aquat. Sci. 41: 766-776.

LIKENS, E.G., R.F. WRIGHT, J. GALLOWAY and T. BUTLER. 1979. Acid rain. Sci. Am. 241: 43-51.

LOCKE, B.A. and L.D. SCOTT. 1986. Ontario Ministry of the Environment Data Report DR 86/4: 80 pp.

MACKAY, R.J. and K.E. KERSEY. 1985. A preliminary study of aquatic insect communities and leaf decomposition in acid streams near Dorset, Ontario. Hydrobiol. 122: 3-11.

MINSHALL, G.W. and J.N. MINSHALL. 1978. Further evidence on the role of chemical factors in determining the distribution of benthic invertebrates in the River Duddon. Arch. Hydrobiol. 83: 324-355.

ODEN, S. 1976. The acidity problem--an outline of concepts. Water Soil and Air Pollut. 6: 137-166.

RADDUM, G.G. and A. FJELLHEIM. 1984. Acidification and early warning organisms in freshwater in Western Norway. Appl. Limnol. 2: 1973-1980.

ROSS, D.H. and R.W. MERRITT. 1978. The larval instars and population dynamics of five species of black flies (Diptera: Simuliidae) and their responses to selected environmental factors. Can. J. Zool. 56(8): 1633-1642.

ROSS, D.H. and R.W. MERRITT. 1987. Factors affecting larval black fly distributions and population dynamics. In: Black flies. Ecology, population management and annotated world list. K.C. KIM and R.W. MERRITT (eds.). Pennsylvania State University, Penn. pp. 90-108.

SERVOS, M.R. and G.L. MACKIE. 1986. The effect of short-term acidification during spring snowmelt on selected Mollusca in south-central Ontario. Can. J. Zool. 64: 1690-1695.

SHARPE, W.E., T.G. PERLIC, W.M. TZILKOWSKI and W.G. KIMMEL. 1987. Status of headwater benthic insect populations in an area of high hydrogen ion and sulfate deposition. Northeastern Environmental Science 6: 23-30.

SIMPSON, K.W., R.W. BODE and J.R. COLQUHOUN. 1985. The macroinvertebrate fauna of an acid-stressed headwater stream system in the Adirondack Mountains, New York. Freshwater Biol. 15: 671-681.

STONE, A. and H.A. JAMNBACK. 1955. The black flies of New York State (Diptera: Simuliidae). New York State Mus. Bull. 349: 1-144.

STONER, J.H., A.S. GEE and K.R. WADE. 1984. The effects of acidification on the ecology of streams in the Upper Tywi catchment in West Wales. Environmental Pollution. 35: 125-157.

SUTCLIFFE, D.W. and T.R. CARRICK. 1973. Studies on mountain streams in the English Lake District I. pH, calcium and the distribution of invertebrates in the River Duddon. Freshwater Biol. 3: 437-462.

WOOD, D.M., B.V. PETERSON, D.M. DAVIES and H. GYORKOS. 1963. The black flies (Diptera: Simuliidae) of Ontario. Part II: Larval identification with descriptions and illustrations. Proc. Entomol. Soc. Ont. 93: 99-129.

WRIGHT, R.F., T. DALE, E.T. GJESSING, G.R. HENDRY, A. HENRIKSEN, M. JOHANNESSEN and I.P. MUINZ. 1976. Impact of acid precipitation on freshwater ecosystems in Norway. Water Air and Soil Pollut. 6: 483-499.

ZISCHKE, J.A., J.W. ARTHUR, KJ NORDLIE, R.O. HERMANUTZ, D.A. STANDEN and T.P. HENRY. 1983. Acidification effects on macroinvertebrates and fathead minnows (*Pimephales promelas*) in outdoor experimental channels. Water Res. 17: 47-63.

Table 1. Experimental design. Three sets (= runs) each with two experiments were conducted. In Run 2 the experiment at pH 3.5 was discarded because of technical problems. 6 channels (3 acidified, 3 control) x 3 chambers/channel x 50 blackfly larvae/emergence chamber = 900 larvae total per experiment.

Run	Starting Date, 1987	Duration (days)	Target pH Acidified Channels	pH (s) Control Channels	
1	29 April	4	4.0	5.3	(0.6)
	4 May	4	3.5	5.7	(0.1)
2	19 May	3	4.0	5.8	(0.0)
	22 May	4	3.5	5.8	(0.0)
3	12 June	3	4.0	5.8	(0.0)
	15 June	3	3.5	5.9	(0.0)

Table 2. Percent survival of *Cnephia* and *Simulium* spp. in the acidification experiments. Survival was pooled over all emergence chambers in the control or acidified channels. Contingency table (2x2) Chi-square probabilities are given; (*) denotes significantly different survival in the control and acidified channels at alpha = 0.05.

Experiment	Species	Percent Survival (N)		x^2
		Control	Acidified	Probability
Run 1, pH 4.0	*C. dacotensis*	59.8 (122)	51.1 (94)	0.1-0.5
	S. venustum	47.4 (215)	29.5 (261)	<0.001*
Run 1, pH 3.5	*C. dacotensis*	43.1 (130)	41.3 (80)	>0.9
	S. decorum	44.4 (18)	20.0 (10)	0.1-0.5
Run 2, pH 4.0	*S. decorum*	71.4 (7)	25.0 (16)	0.1-0.5
	S. venustum	75.6 (123)	71.8 (231)	0.5-0.9
	S. verecundum	92.9 (85)	89.1 (101)	0.5-0.9
Run 3, pH 4.0	*S. decorum*	42.3 (156)	42.1 (126)	>0.9
	S. venustum	40.6 (106)	26.3 (136)	0.025-0.05*
	S. vittatum	40.3 (77)	18.9 (90)	0.001-0.005*
Run 3, pH 3.5	*S. decorum*	50.8 (65)	6.0 (84)	<0.001*
	S. venustum	59.5 (76)	2.5 (157)	<0.001*
	S. verecundum	79.3 (62)	39.1 (46)	<0.001*
	S. vittatum	49.1 (108)	8.0 (100)	<0.001*

Table 3. Chemical analysis of water samples from the experimental acidification channels. Data represent arithmetic means (with standard deviations) of samples from three acid or three control channels at the start and end of each month. Cd, Cu, Pb and Zn were measured on water collected from one randomly chosen acidified and control channel at the end of each run. (<) indicates a value at the minimum detection level.

Run	Channels	Sample	pH	Alkalinity (mg/L)	Aluminum (µg/L)	Calcium (mg/L)	Conductivity @ 25°C (µS/cm)	DIC (mg/L)	DOC (mg/L)	Sulphate (mg/L)	Cadmium (µg/L)	Copper (µg/L)	Lead (µg/L)	Zinc (µg/l)
1	control	start	5.31 (0.63[1])	-0.34 (1.01)	27.33 (0.58)	1.84 (0.03)	21.17 (0.06)	0.53 (0.01)	1.83 (0.32)	6.00 (0.22)				
		end	5.65 (0.08)	0.19 (0.05)	28.67 (2.31)	1.81 (0.07)	21.43 (0.15)	0.60 (0.01)	1.73 (0.06)	6.30 (0.20)	0.008	<0.30	<0.04	5.26
	acid	start	4.94 (0.07)	-0.66 (0.15)	28.33 (1.53)	1.86 (0.01)	25.63 (0.25)	0.52 (0.01)	1.60 (0.00)	6.75 (0.00)				
		end	3.51 (0.02)	-20.00 (0.00)	39.33 (13.58)	2.22 (0.14)	131.10 (61.37)	0.57 (0.01)	1.77 (0.06)	24.13 (0.25)	<0.010	<0.30	<0.04	6.11
2	control	start	5.79 (0.02)	0.52 (0.04)	19.33 (2.31)	1.63 (0.06)	20.97 (0.25)	0.61 (0.01)	1.53 (0.06)	6.10 (0.09)				
		end	5.78 (0.01)	0.49 (0.03)	24.00 (1.73)	1.73 (0.06)	21.97 (0.29)	0.76 (0.01)	1.70 (0.00)	6.16 (0.09)	0.076	<0.30	0.21	3.47
	acid	start	4.01 (0.01)	-5.13 (0.12)	22.33 (1.53)	2.10 (0.46)	58.10 (0.10)	0.59 (0.01)	1.53 (0.06)	9.38 (2.80				
		end	3.59 (0.02)	-1.60 (0.10)	27.00 (1.73)	2.60 (0.35)	135.00 (3.61)	0.73 (0.01)	1.80 (0.00)	13.88 (0.84)	0.012	0.66	0.19	3.52
3	control	start	5.84 (0.02)	0.53 (0.08)	18.33 (0.58)	1.73 (0.12)	21.10 (0.10)	0.66 (0.03)	1.93 (0.06)	6.18 (0.06)				
		end	5.91 (0.01)	0.78 (0.03)	18.00 (0.00)	1.80 (0.10)	21.00 (0.26)	0.63 (0.01)	2.07 (0.06)	5.85 (0.00)	<0.010	<0.30	-	3.09
	acid	start	4.14 (0.01)	-4.30 (0.00)	20.33 (0.58)	2.20 (0.17)	53.47 (1.08)	0.61 (0.01)	2.07 (0.06)	10.70 (0.10)				
		end	3.55 (0.01)	-15.33 (0.58)	23.67 (0.58)	2.20 (0.26)	141.33 (0.52)	0.59 (0.02)	2.23 (0.15)	22.65 (1.58)	<0.010	1.36	-	4.06

Table 4. Percentages of larvae (L), abnormal half-pupated forms (1/2 P), pupae (P) and adults (Ad) of *Cnephia* and *Simulium* spp. remaining of the total number (N) in the control (C) and acidified (A) channels at the end of the experimental acidifications. The *'s indicate life stages with significantly greater survival in the control channels. The **'s indicate life stages with significantly greater survival in the acidified channels. TRT = treatment.

		Run 1, pH 4.0					Run 1, pH 3.5					Run 2, pH 4.0				
Species	TRT	N	L	1/2P	P	Ad	N	L	1/2P	P	Ad	N	L	1/2P	P	Ad
C. dacotensis	C	122	96	1	2	0	130	100	0	0	0	-	-	-	-	-
	A	94	100	0	0	0	0	80	100	0	0	0	-	-	-	--
S. decorum	C	-	-	-	-		18	94	0	6	0	7	57	0	43	0
	A	-	-	-	-		10	100	0	0	0	16	75	19	6	0
S. venustum	C	215	92	7	1	0	-	-	-	-	-	123	91	7	2	0
	A	261	91*	9	0	0	-	-	-	-	-	231	91	9	0	0
S. verecundum	C	-	-	-	-	-	-	-	-	-	-	85	97	2	1	0
	A	-	-	-	-	-	-	-	-	-	-	101	97	3	0	0
S. t/v/v[1]	C	35	8	46	46	-	16	0	50	50	0	77	18	9	73**	
	A	24	0	58	42	-	8	0	38	62	0	46	4	35	61	

(Cont.)

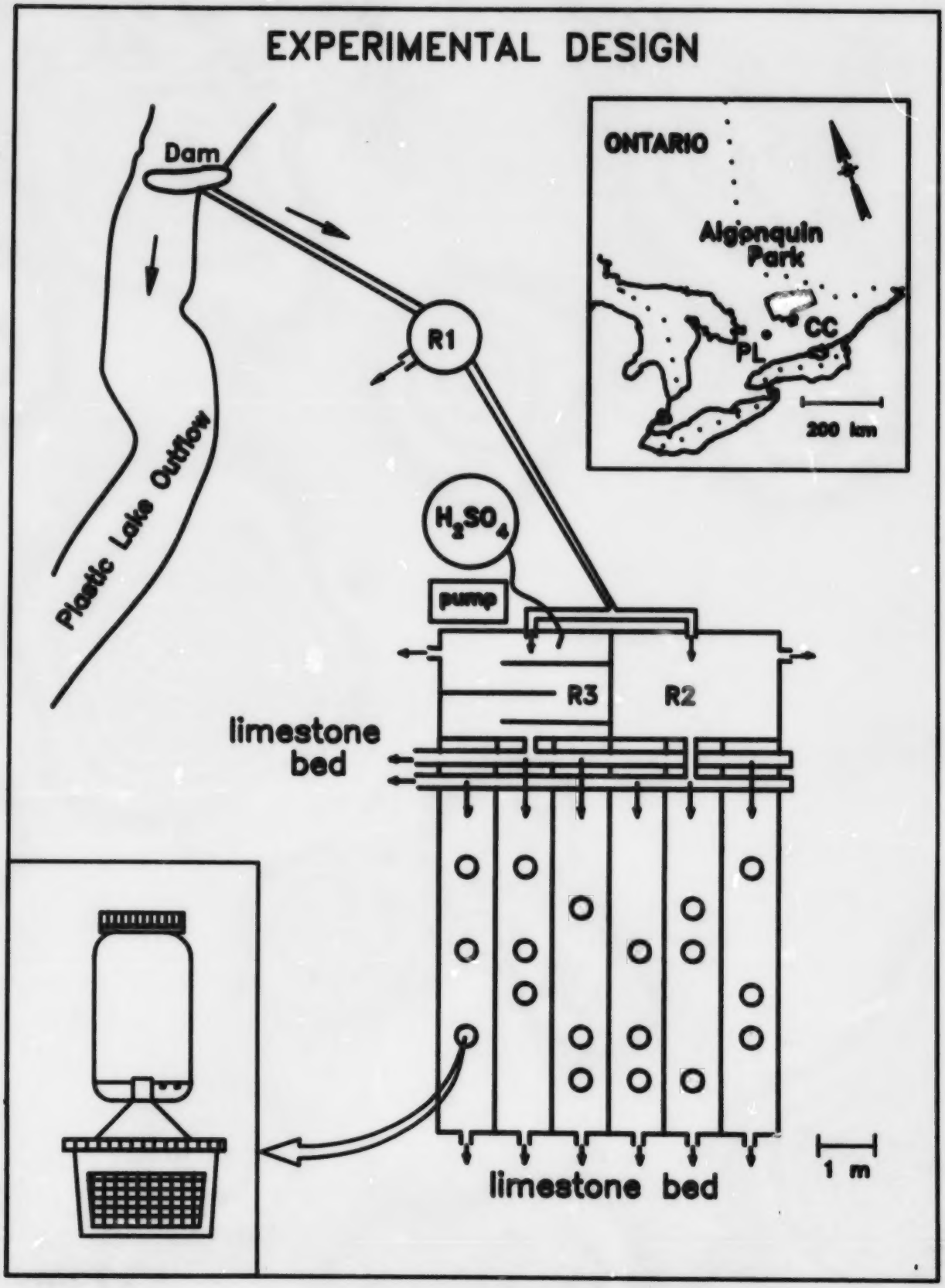
Table 4. (Cont.)

Species	TRT	Run 3, pH 4.0					Run 3, pH 3.5				
		N	L	1/2P	P	Ad	N	L	1/2P	P	Ad
C. dacotensis	C	-	-	-	-		-	-	-	-	-
	A	-	-	-	-		-	-	-	-	-
S. decorum	C	156	35	8	57	1	65	52	3	31	14
	A	126	48	30	21	1	84	74	16	6	5
S. venustum	C	106	64	11	25	0	76	86	13	1	0
	A	136	81	15	4	0	157	75	22	3	0
S. verecundum	C	-	-	-	-	-	62	6	8	72	15
	A	-	-	-	-	-	46	39	15	35	11
S. vittatum	C	77	65	9	26	0	108	49*	8	39	4
	A	90	76	18	6	0	100	71	17	10	2
S. t/v/v[1]	C	48	0	0	100	0	107	0	8	89*	3
	A	36	0	39	61	0	35	0	49	49	3

[1]combined group of inseparable *Simulium tuberosum*, *S. venustum*, and *S. verecundum*.

FIGURES

Figure 1 Stream channels and cylindrical containers used to measure survival of larvae and pupae and emergence of adult blackflies in the experimental acidification studies. Channels were located on Plastic Lake Outflow (PL) in Haliburton Co., Ontario and blackfly larvae were collected from Costello Creek (CC), Algonquin Park, Ontario (inset). See text for details on construction and operation. Arrows indicate water flow.


EXPERIMENTAL DESIGN
Dam
Plastic Lake Outflow
R1
ONTARIO
Algonquin Park
CC
PL
200 km
H_2SO_4
pump
R3
R2
limestone bed
limestone bed
1 m